A34586 (070050.1668)

In re: United States Patent Application by Fisher *et al.*

Serial No. 09/684,310 Examiner: Yu, Misook

Filed: August 25, 2000 Group Art Unit: 1642

Title: PROGRESSION SUPPRESSED GENE 13 (PSGen-13) AND USES THEREOF

DECLARATION OF DR. PAUL B. FISHER

1. I, Dr. Paul B. Fisher, am an expert in cell biology, gene identification and cancer gene therapy. I currently am a Professor of Clinical Pathology and Director of Neuro-oncology with joint appointments in the Departments of Pathology, Urology and Neurosurgery and am the Michael and Stella Chernow Urological Cancer Research Scientist at the College of Physicians and Surgeons, Herbert Irving Comprehensive Cancer Center, Columbia University, New York, New York. I have a Ph.D. in cell biology, virology and somatic cell genetics. I have held academic positions for more than 20 years. I have as of the present time published more than 200 peer-reviewed articles in prestigious scientific journals, been commissioned to write several review articles and invited to deliver national and international seminars in my area of expertise. I am the recipient of several federally and privately funded research grants. I have served on scientific review committees for various Federal, private not-for-profit and international agencies including the National Institutes of Health, the CaPCure Foundation, The Samuel Waxman Cancer Research Foundation, The California Breast Cancer Research Foundation, The Dutch Cancer Research Society, the Italian Cancer Research Foundation etc. I hold a number of patents. A copy of my curriculum vitae is attached as Exhibit A.

2. I am a co-inventor of the above-identified Patent Application.

3. The experiments described in the specification of the above-identified application were performed under my direction.

4. I understand that the Examiner has found that certain claims in the instant application are not enabled. In response, I offer the following information based on experimental findings performed under my supervision:

A. I would like to draw the Examiner's attention to Exhibit B. The experimental data shown in Exhibit B (Figure 1) demonstrates the effect of PSGen13 expression on a pre- formed tumor comprising DU-145 human prostate cancer cells utilizing an adenoviral vector Ad.PSGen13. Two million DU-145 cells were injected subcutaneously into the flanks of fifteen male athymic nude mice which were then divided into three sets of five animals each. Tumors formed at the site of injection, and once these attained a volume of approximately 75 mm^3, intratumoral injection with adenovirus preparations was performed. One set of animals were untreated (control), another set was injected with a control adenovirus (Ad.vec) and the third set injected with an adenovirus expressing PsGen13. Injections where applicable were performed totally seven times over a three week period at a dosage of $1x10^8$ pfu/100_l virus. At the end of six weeks, tumors treated with PSGen13 were approximately four times smaller in volume than control or Ad.vec treated tumors. Thus treatment of tumors with PsGen13 gene product resulted in inhibition of tumor cell growth as reflected by a smaller tumor volume in the PSGen13 treated samples.

B. This data is a further experimental demonstration of data provided in the specification showing the inhibitory activity of the PSGen13 gene on cancer cells and additionally demonstrates the activity of PSGen13 *in vivo*. In Exhibit B, the gene was delivered to a pre-formed tumor in nude mouse xenografts resulting in a reduction in tumor volume compared to simultaneously performed control sets. The treatment protocol described in Exhibit B would be considered as a form of cancer gene therapy.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from the above-captioned patent application.

Paul B. Fisher 4/14/05

Dr. Paul B. Fisher Date

CURRICULUM VITAE:

Dr. Paul B. Fisher

CURRICULUM VITAE:

Dr. Paul B. Fisher

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Paul B. Fisher	**Professor**

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Hunter College of CUNY, NY	B.A.	1968	Biology/Chemistry
Herbert H. Lehman College of CUNY, NY	M.A.	1971	Genetics
Rutgers University, NJ	M.PH.	1973	Cell Biology, Virology
Waksman Institute of Microbiology	Ph.D.	1974	& Somatic Cell Genetics

Professional Experience:

1987-Present	**Michael and Stella Chernow Urological Cancer Research Scientist**, Departments of Pathology and Urology, Columbia University, College of Physicians & Surgeons, NY, NY 10032
1987-Present	**Adjunct Professor and Visiting Scholar**, New York University, NY, NY 10003
1988-Present	**Director of Neuro-Oncology Research**, Department of Neurological Surgery, Columbia University, College of Physicians & Surgeons, NY, NY 10032
1991-Present	**Professor of Clinical Pathology**, Department of Pathology, Columbia University, College of Physicians and Surgeons, NY, NY 10032

Editorial and Association Boards: Archives of AIDS Research (Associate Editor); Cancer Biology and Therapy (Editorial Board); Cancer Research (Associate Editor); In Vivo (Associate Editor); International Institute of Cancer Research (Scientific Advisory Board); International Journal of Oncology (Associate Editor); International Society of Cancer Gene Therapy (Council Member); International Society of Differentiation (Board of Directors); Journal Experimental Therapeutics & Oncology (Associate Editor); Journal Experimental & Clinical Cancer Research (Associate Editor); Mechanisms of Differentiation (Series Editor; CRC Press); Molecular & Cellular Differentiation (Editor-in-Chief; CRC Press); Urology (Expert Reviewer); **Consultantships**: Project and Site Visit Reviewer for Health Effects Division of DOE; Ad Hoc Reviewer Chemical Pathology Study Section; **Grant Reviewer**: NSF, NCI, DOE, New Jersey Commission on Cancer Research, California Breast Cancer Foundation and Ontario Ministry of Health, Canada.

Selected Publications (from a Total of 300):

1. Jiang, H., J. J. Lin, Z.-z. Su, N. I. Goldstein and P. B. Fisher. Subtraction hybridization identifies a novel melanoma differentiation associated gene, *mda-7*, modulated during human melanoma differentiation, growth and progression. *Oncogene* 11: 2477-2486, 1995.
2. Jiang, H., Z.-z. Su, J. J. Lin, N. I. Goldstein, C. S. H. Young and P.B. Fisher. The melanoma differentiation associated gene *mda-7* suppresses cancer cell growth. *Proc. Natl. Acad. Sci. USA* 93: 9160-9165, 1996.
3. Su, Z.-z., Y. Shi and P.B. Fisher. Subtraction hybridization identifies a progression elevated gene *PEG-3* with sequence homology to a growth arrest and DNA damage inducible gene. *Proc. Natl. Acad. Sci. USA* 94: 9125-9130, 1997.
4. Su, Z.-z., M.T. Madireddi, J.J. Lin, C.S.H. Young, S. Kitada, J.C. Reed, N.I. Goldstein and P.B. Fisher. The cancer growth suppressor gene *mda-7* selectively induces apoptosis in human breast cancer cells and inhibits tumor growth in nude mice. *Proc. Natl. Acad. Sci. USA* 95: 14400-14405, 1998.
5. Kang, D.-c., R. La France, Z.-z. Su and P.B. Fisher. Reciprocal subtraction differential RNA display (RSDD): an efficient and rapid procedure for isolating differentially expressed gene sequences. *Proc. Natl. Acad. Sci. USA* 95: 13788-13793, 1998.
6. Su, Z.-z., N.I. Goldstein, H. Jiang, M.-N. Wang, G.J. Duigou, C.S.H. Young and P.B. Fisher. *PEG-3*, a non-transforming progression gene, is a positive regulator of cancer aggressiveness and angiogenesis. *Proc. Natl. Acad. Sci. USA* 96: 15115-15120, 1998.
7. Huang, F., J. Adelman, H. Jiang, N.I. Goldstein and P.B. Fisher. Identification and temporal expression pattern of genes modulated during irreversible growth arrest and terminal differentiation in human melanoma cells. *Oncogene* 18: 3546-3552, 1999.
8. Huang, F., J. Adelman, H. Jiang, N.I. Goldstein and P. B. Fisher. Differentiation induction subtraction hybridization (DISH): an approach for cloning genes differentially expressed during growth arrest and terminal differentiation in human melanoma cells. *Gene* 236: 125-131, 1999.
9. Gopalkrishnan, R. V., K. A. Christiansen, N. I. Goldstein, R. A. DePinho and P. B. Fisher. Use of the human EF-1α promoter for expression can significantly increase success in establishing stable cell lines with consistent expression: a study using the tetracycline inducible system in human cancer cells. *Nucl. Acids Res.* 27: 4775-4782, 1999.
10. Madireddi, M. T., Su, Z.-z., C.S.H. Young, N.I. Goldstein and P.B. Fisher. *Mda-7*, a novel melanoma differentiation associated gene with promise for cancer gene therapy. *Adv. Exptl. Med. Biol.* 465: 239-261, 2000.
11. Madireddi, M.T., P. Dent and P.B. Fisher. Regulation of *mda-7* gene expression during human melanoma differentiation. *Oncogene* 19: 1362-1368, 2000.

12. Madireddi, M.T., P. Dent and P.B. Fisher. AP-1 and C/EBP transcription factors contribute to *mda-7* gene promoter activity during human melanoma differentiation. *J. Cell. Physiol.* 185: 36-46, 2000.
13. Jiang, H., D.-c. Kang, D. Alexandre and P. B. Fisher. RaSH, A rapid subtraction hybridization approach for identifying and cloning differentially expressed genes. *Proc. Natl. Acad. Sci. USA* 97: 12684-12689, 2000.
14. Su, Z.-z., Y. Shi and P. B. Fisher. Cooperation between AP1 and PEA3 sites within the progression elevated gene-3 (PEG-3) promoter regulate basal and differential expression of PEG-3 during progression of the oncogenic phenotype in transformed rat embryo cells. *Oncogene* 19: 3411-3421, 2000.
15. Kang, D.-c., H. Jiang, Q. Wu, S. Pestka and P. B. Fisher. Cloning and characterization of human ubiquitin-processing protease-43 from terminally differentiated human melanoma cells using a rapid subtraction hybridization protocol RaSH. *Gene* 267: 233-242, 2001.
16. Leszczyniecka, M., T. Roberts, P. Dent, S. Grant and P.B. Fisher. Differentiation therapy of cancer: basic science and clinical applications. *Pharmacology and Therapeutics* 90:105-156, 2001.
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18. Su, Z.-z., Y. Shi, R. Friedman, L. Qiao, D. Hinman, P. Dent and P. B. Fisher. PEA3 sites within the progression elevated gene-3 (*PEG-3*) promoter and mitogen activated protein kinase contribute to differential *PEG-3* expression in Ha-ras and v-raf oncogene transformed rat embryo fibroblast cells. *Nucl. Acids Res.* 29: 1661-1671, 2001.
19. Su, Z.-z., I.V. Lebedeva, R.V. Gopalkrishnan, N.I. Goldstein, C. A. Stein, J.C. Reed, P. Dent and P.B. Fisher. A combinatorial approach for selectively inducing programmed cell death in human pancreatic cancer cells. *Proc. Natl. Acad. Sci. USA* 98: 10332-10337, 2001.
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21. Gopalkrishnan, R.V., D.-c. Kang and P.B. Fisher. Molecular markers and determinants of human prostate cancer metastasis. *J. Cell. Physiol.* 189: 245-256, 2001.
22. Pillutla, R.C., A.J. Blume, N.I. Goldstein and P.B. Fisher,. Target validation and drug discovery using genomic and display technologies. *Expert Opinion in Therapeutic Targets* 6: 517-532, 2002.
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41. Lebedeva, I.V., D. Sarkar, Z.-z. Su, S. Kitada, P. Dent, C.A. Stein, J.C. Reed and P.B. Fisher. Bcl-2 and Bcl-x_L differentially protect human prostate cancer cells from induction of apoptosis by melanoma differentiation associated gene-7, *mda*-7/IL-24. Oncogene 22: 8758-8773.
42. Lebedeva, I.V., Z.-z. Su, D. Sarkar, S. Kitada, P. Dent, S. Waxman, J.C. Reed and P.B. Fisher. Melanoma differentiation associated gene-7, *mda*-7/IL-24, promotes apoptosis in prostate cancer cells by promoting mitochondrial dysfunction and inducing reactive oxygen species. Cancer Res. 63: 8138-8144, 2003.
43. Kang, D.-c., R. V. Gopalkrishnan, L. Lin, K. Valerie, S. Pestka and P.B. Fisher. Expression analysis and genomic characterization of human melanoma differentiation associated gene-5, *mda*-5: a novel type I interferon apoptosis-inducing gene. Oncogene, 23:1789-1800, 2004.
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Research Projects Active and Completed During the Last 3 Years:

"Analysis of Progression of the Transformed Phenotype" (Active)

Principal Investigator: Fisher, P.B.
Type/Grant No.: 1 R01 CA35675-19

Funding Agency: NIH/NCI
Period: 04/01/84 to 11/30/07

Determine the functional significance of a novel gene progression elevated gene-3 (PEG-3) in cancer progression.

"Mda-7: Novel Cancer Therapeutic Gene" (Active)

Principal Investigator: Fisher, P.B.
Type/Grant No.: 1 R01 CA97318-03

Funding Agency: NIH/NCI
Period: 10/01/02 to 9/30/07

Mechanism of action of the novel cancer-specific apoptosis-inducing gene *mda*-7/IL-24. This project focuses on the role of *mda*-7/IL-24 in inducing apoptosis selectively in melanoma with emphasis on interacting proteins and the role of cell surface receptors in mediating *mda*-7 activity.

"Novel Approaches for Pancreatic Cancer Therapy" (Active)

Principal Investigator: Fisher, P. B.
Type/Grant No.: 1 R01 CA098712-02

Funding Agency: NIH/NCI
1/21/03 to 1/01/08

EXHIBIT B

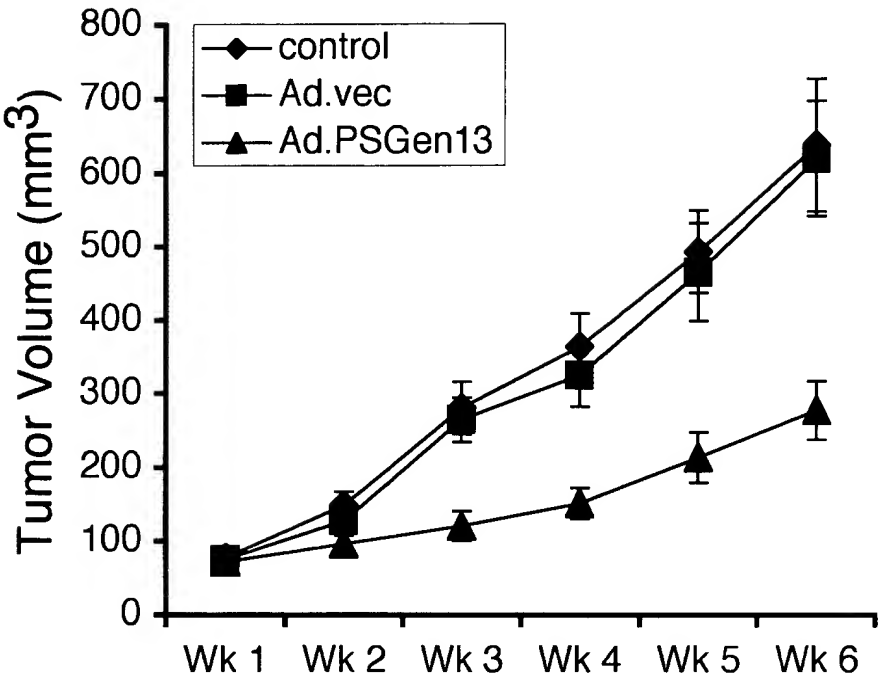

FIGURE 1: Ad.PSGen13 inhibits the growth of DU-145 tumors *in vivo*. Subcutaneous tumor xenografts from DU-145 cells were established in athymic nude mice in the left flank and the tumors were injected with PBS (control) or with the indicated Ad for 3 weeks (total of seven injections). The figure shows the tumor volume measured as described in materials and methods. The data represent mean $\pm$ S.D. with at least 5 mice in each group.

Materials and Methods: DU-145 human prostate carcinoma cells ($2x10^6$) were injected subcutaneously in 100 µl of PBS in the left flank of male athymic nude mice ($NCR^{nu/nu}$; 4 weeks old; ~20 g body weight). After the establishment of visible tumors of ~75 mm^3, requiring ~4-5 days, intratumoral injections of different Ad were given at a dose of $1x10^8$ pfu in 100 µl. The injections were given 3 times a week for the first week and then twice a week for two more weeks to a total of seven injections. At least 5

animals were used per experimental point. Tumor volume was measured twice weekly with a caliper and calculated using the formula $\pi/6$ x larger diameter x (smaller diameter)^2. At the end of the experiment the animals were sacrificed and the tumors were removed and weighed.

Conclusion: Intratumoral injection of Ad.PSGen13 in established DU-145 human prostate cancer xenografts in nude mice significantly inhibited the tumor growth when compared to that of control or Ad.vec (control empty adenovirus) injections. At the end of the experiments (6 weeks after the establishment of the tumors), the tumor volume in control and Ad.vec-injected animals were 637.8 ± 89.33 mm^3 and 619.62 ± 77.98 mm^3, respectively while that in Ad.PSGen13-injected animals were 277.56 ± 39.78 mm^3 indicating that Ad.PSGen13 injection resulted in significant inhibition of tumor growth.